

Please see <http://www.fda.gov/cder/drug/infopage/heparin/default> for updated information on screening methods. 7/2008

Impurity Evaluation of Heparin Sodium by ^1H -NMR Spectroscopy

Instrument:

500 MHz NMR, less than 500MHz can be used if appropriately qualified material shows good separation between the N-acetyl protons of over sulfated chondroitin sulfate, dermatan sulfate and heparin sodium

Reagents:

Solvent: D_2O (Deuterated water)

Internal reference standard: TSP (tri-methyl-silyl propionate, sodium salt) to be referenced at 0.00 ppm.

Preparation of Test solutions:

Weigh between 10 and 40 mg of heparin sodium into a 5 mm NMR tube and dissolve in 0.6 ml of D_2O spiked with 0.05 to 0.10% by weight TSP. Sample may require several minutes of constant agitation to dissolve.

^1H -NMR analysis:

Collect ^1H -NMR spectrum on a 500 MHz NMR instrument.

Spectral parameters should include no less than 16 transients, 90 degree pulse width, acquisition time of at least one second, time between transients of 20 seconds and a spectral window of 8000 hz. The number of transients should be adjusted until the signal-to-noise is at least 200/1 in the region near 2 ppm.

The sample should be run at 25 °C.

Criteria:

The N-acetyl protons of heparin should show a single peak at 2.04 ppm ($\pm 0.02\text{ppm}$). A small dermatan sulfate peak, corresponding to N-acetyl protons of dermatan sulfate, may show near 2.08 ppm. **No peak should be visible at 2.15 ± 0.02 ppm.**

